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UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity) *Only for new nonprovisional applications under 37 C.F.R. 1.53(b)*	Docket No.: **2874-B** Express Mail Label No. **EL591095097US**

TO THE ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

METHOD OF INHIBITING OSTEOCLAST ACTIVITY

and invented by:

Dirk M. Anderson, residing at Seattle, Washington and Laurent J. Galibert residing at Seattle, Washington.

If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP)

of prior application No.: PCT/US99/10588

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification including claims and abstract (13 pages total)
3. ☐ Drawing(s); Number of Sheets
4. ☒ Oath or Declaration
   a. ☒ Newly executed
   b. ☐ Copy from a prior application (37.C.F.R. 1.63(d)) (*for continuation/divisional application only*)
   c. ☐ With Power of Attorney ☒ Without Power of Attorney
   d. ☐ DELETION OF INVENTOR(S)

      Signed statement attached deleting inventor(s) named in prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation by Reference (usable if Box 4b is checked)

   The entire disclosure of the prior application from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Computer Program in Microfiche (Appendix)
7. ☒ Nucleotide and/or Amino Acid Sequence Submission
   a. ☒ Paper copy
      ☐ Pages - _____ of specification
      ☒ Separately numbered pages 1 - 13
   b. ☐ Computer Readable Copy
   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy
   d. ☒ Statement under 37 C.F.R. 1.821(e) in lieu of Computer Readable Copy

UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)	Docket No.: 2874-B

Accompanying Application Parts

8. ☒ Assignment
   a. ☐ Executed original Assignment and Recordation Form enclosed
   b. ☐ Prior application is assigned of record to Immunex Corporation (reel _____ frame_____)
9. ☒ 37 C.F.R. 3.73(B) Statement (*when there is an assignee*)
10. ☒ Preliminary Amendment
11. ☒ Acknowledgment postcard
12. ☒ Certificate of Mailing by Express Mail (Label No.: EL591095097US)
13. ☐ Certified Copy of Priority Document(s) (*if foreign priority is claimed*)
14. ☐ Additional Enclosures (*please identify below*):

Fee Calculation and Transmittal

CLAIMS AS FILED (after any Preliminary Amendment submitted herewith)					
For	**# Filed**	**# Allowed**	**# Extra**	**Rate**	**Fee**
Total Claims	24	- 20 =	4	x $18.00	**$72.00**
Indep. Claims	3	- 3 =	0	x $80.00	**$0.00**
Multiple Dependent Claims (check if applicable) ☐					**$0.00**
				BASIC FEE	**$710.00**
OTHER FEE (*specify purpose*)					**$0.00**
				TOTAL FILING FEE	**$782.00**

☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 09-0089 as described below. A copy of this sheet is enclosed.

☒ Charge the amount of $782.00 as a filing fee.

☒ Credit any overpayment.

☒ Charge any additional fees required under 37 C.F.R. 1.16 and 1.17.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	Dirk M. Anderson and Laurent J. Galibert	Docket No.:	2874-B
		Group Art Unit:	Unknown
Serial No:	--to be assigned--	Examiner:	Unknown
Filed:	November 3, 2000		

For: METHOD OF INHIBITING OSTEOCLAST ACTIVITY

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Prior to examining the above-referenced patent application, please enter the following amendments into the application:

Between the Title and the Technical Field of the Invention, please insert the following paragraph:

- - This application is a continuation of PCT/US99/10588, filed May 13, 1999, which claims the benefit of priority from U.S. provisional applications 60/085,487, filed May 14, 1998, and 60/110,836, filed December 3, 1998, and is a continuation-in-part of United States patent application 08/966,139 (now U.S. 6,017,729), filed December 22, 1997, which claims the benefit of priority from U.S. provisional applications 60/064,671, filed October 14, 1997; 60/077,181, filed March 7, 1997; and 60/059,978, filed December 23, 1996. - -

In the Specification:

At page 3, line 31, please delete [a nucleotide]

At page 3, at line 7, between "NO:2." and "Moreover" please insert:

- - Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a 'spacer' region, which is not necessary for ligand binding. In RANK, the amino acids between

196 and 213 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 213 and 196 of SEQ ID NO:2, although other amino acids in the spacer region may be utilized as a C-terminus. In muRANK, the amino acids between 197 and 214 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 214, and 197 of SEQ ID NO:5, although other amino acids in the spacer region may be utilized as a C-terminus. - -

At page 4, line 28, please delete [NO:6] and substitute therefor - - NO:5 - -

At page 7, between lines 5 and 6, please insert the following paragraph:

- - Soluble forms of RANK and other RANK antagonists such as antagonistic monoclonal antibodies can be administered for the purpose of inhibiting RANK-induced induction of NF-κB activity. NF-κB is a transcription factor that is utilized extensively by cells of the immune system, and plays a role in the inflammatory response. Thus, inhibitors of RANK signalling will be useful in treating conditions in which signalling through RANK has given rise to negative consequences, for example, toxic or septic shock, or graft-versus-host reactions. They may also be useful in interfering with the role of NF-κB in cellular transformation. Tumor cells are more responsive to radiation when their NF-κB is blocked; thus, soluble RANK (or other antagonists of RANK signalling) will be useful as an adjunct therapy for disease characterized by neoplastic cells that express RANK. - -

In the claims:

1. A method of [regulating] inhibiting osteoclast [activity] generation, the method comprising [causing] administering to a patient in need thereof a therapeutic composition comprising a recombinant soluble RANK [to bind RANKL] polypeptide.

In Claim 2(a), line 3, please delete [62] and substitute therefor - - 2 - -

In Claim 2(b), line 2, please delete [NO:6] and substitute therefor - - NO:5 - -

In Claim 2(b), line 3, please delete [NO:6] and substitute therefor - - NO:5 - -

5. (once amended) A method of ameliorating effects of excess bone loss, comprising administering a soluble RANK polypeptide composition to an individual at risk for excess bone loss[, and allowing the soluble RANK to bind RANKL and inhibit binding thereof to cells expressing RANK].

6. (once amended) The method of claim 5, wherein the individual is at risk from or suffers from a condition selected from the group consisting of osteoporosis, [Pagett's] Paget's disease, [and] bone cancer, multiple myeloma, melanoma, breast cancer and cancers associated with hypercalcemia.

In Claim 7(a), line 3, please delete [62] and substitute therefor - - 2 - -

In Claim 7(b), line 2, please delete [NO:6] and substitute therefor - - NO:5 - -

In Claim 7(b), line 3, please delete [NO:6] and substitute therefor - - NO:5 - -

In Claim 10(a), line 3, please delete [62] and substitute therefor - - 2 - -

In Claim 10(b), line 2, please delete [NO:6] and substitute therefor - - NO:5 - -

In Claim 10(b), line 3, please delete [NO:6] and substitute therefor - - NO:5 - -

Please add the following new claims:

13. A method of ameliorating the effects of excess bone loss comprising administering to a patient in need thereof a therapeutic composition comprising a recombinant soluble RANK polypeptide, wherein said patient suffers from a condition selected from the group consisting of squamous cell carcinoma, lung cancer, prostate cancer, hematologic cancer, head and neck cancer and renal cancer.

14. The method of claim 13, wherein the soluble RANK polypeptide is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:2, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:5, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:5, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) a DNA capable of hybridizing to the DNA of (a) or (b) under stringent conditions, and that encodes a RANK polypeptide that binds RANKL; and

(d) a DNA molecule encoding a fragment of a protein encoded by a DNA of (a), (b) or (c), wherein said fragment binds RANKL.

15. The method of claim 14, wherein the soluble RANK polypeptide is at least about 80% identical in amino acid sequence to native RANK.

16. The method of claim 13, wherein the soluble RANK polypeptide further comprises one or more polypeptides selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG™ tag, a peptide comprising at least about 6 His residues and a leucine zipper.

17. A method according to claim 4, wherein the further polypeptide is selected from the group consisting of an immunoglobulin Fc domain comprising an amino acid sequence as shown in SEQ ID NO:3 and a leucine zipper comprising an amino acid sequence as shown in SEQ ID NO:6.

18. A method according to claim 9, wherein the further polypeptide is selected from the group consisting of an immunoglobulin Fc domain comprising an amino acid sequence as shown in SEQ ID NO:3 and a leucine zipper comprising an amino acid sequence as shown in SEQ ID NO:6.

19. A method according to claim 12, wherein the further polypeptide is selected from the group consisting of an immunoglobulin Fc domain having an amino acid sequence as shown in SEQ ID NO:3 and a leucine zipper having an amino acid sequence as shown in SEQ ID NO:6.

20. A method according to claim 16, wherein the further polypeptide is selected from the group consisting of an immunoglobulin Fc domain having an amino acid sequence as shown in SEQ ID NO:3 and a leucine zipper having an amino acid sequence as shown in SEQ ID NO:6.

21. A method according to claim 1, wherein the soluble RANK polypeptide comprises amino acids 34 through 196 of SEQ ID NO:2.

22. A method according to claim 5, wherein the soluble RANK comprises amino acids 34 through 196 of SEQ ID NO:2.

23. A method according to claim 6, wherein the soluble RANK comprises amino acids 34 through 196 of SEQ ID NO:2.

24. A method according to claim 13, wherein the soluble RANK comprises amino acids 34 through 196 of SEQ ID NO:2.

REMARKS

Claims 1-12 are pending in the application. Claims 1, 2, 5, 6, 7 and 10 have been amended as indicated above, and new Claims 13-24 have been added to the application. The specification has been amended to correct two inadvertent errors, and to add two excerpts derived from a prior application that was originally incorporated by reference into the present application.

The specification is amended at page 3, line 31 to delete the words "a nucleotide" in reference to SEQ ID NO:3. The sequence shown in SEQ ID NO:3 is clearly an amino acid sequence, thus it is self-evident that the words "a nucleotide" represent an inadvertent typing error. Accordingly, the deletion of these two words does not constitute the addition of new matter.

The specification is amended above by the addition of two excerpts taken from patent application 08/966,139 (now U.S. 6,017,729). Inserting these two excerpts into the application does not constitute the addition of new matter because USSN 08/966,139 is incorporated by reference into parent application PCT/US99/10588 (see the specification at page 2, line 35, to page 3, line 1). The first excerpt added herein is found in U.S. 6,027,729 at column 16, lines 14-28, and the second excerpt is found in U.S. 6,027,729, at column 17, line 66 to column 18, line 11, and at column 27, lines 4-13. The excerpts shown above are identical to the text at columns 16-18 of U.S. 6,027,729 except for the actual numbers of the SEQ ID NOS. In U.S. 6,027,729, human and mouse RANK proteins correspond, respectively, to SEQ ID NOS:6 and 14, whereas in the present application, human and mouse RANK proteins correspond, respectively, to SEQ ID NOS:2 and 5. Accordingly, the numbers "6" and "14" in the excerpts added herein were changed to "2" and "5." Since these substitutions ensure that the *same* amino acid sequences are referred to in both documents, they do not constitute the addition of new matter.

The specification is amended at page 4, line 28 to refer to "SEQ ID NO:5" rather than "SEQ ID NO:6." The text surrounding this recitation clearly refers to murine RANK. Taking the specification as a whole into account, it would be readily apparent to one skilled in the art that SEQ ID NO:5, not SEQ ID NO:6, is murine RANK. SEQ ID NO:6, for example, contains only 33 amino acids, thus obviously could not represent a full-length RANK protein (see the specification at page 1, lines 34-35, noting that human RANK has 616 amino acids). Furthermore, the specification at page 1, lines 9-11, refers to two U.S. patent applications that disclose the cloning of RANK and RANKL. One of these is 08/996,139 (now U.S. Patent No.6,017,729), which teaches that murine RANK is a protein having 625 amino acids (see U.S. 6,017,729 at column 26, lines 57-61 and surrounding text). SEQ ID NO:5 in the present application contains 625 amino acids. Moreover, the heading for SEQ ID NO:4 herein, which discloses the same protein as SEQ ID NO:5, identifies the sequence as "muRANK." Thus, this amendment merely corrects a self-evident error and does not constitute the addition of new matter.

Claims 1(a), 7(a) and 10(a) are amended above to refer to "SEQ ID NO:2" rather than "SEQ ID NO:62." The need for this correction is self-evident from the first two lines of these claims, which refer to "an amino acid sequence as set forth in SEQ ID NO:2;" it would be readily apparent to one skilled in the art that the second part of Claims

1(a), 7(a) and 10(a) are meant to refer back to the same sequence that was recited in the initial part of these claims. Furthermore, the sequence listing discloses only six sequences. Thus, it is self-evident that the reference to "NO:62" is an inadvertent error and that the correct sequence listing is SEQ ID NO:2. Accordingly, these amendments do not constitute the addition of new matter.

Claims 1(b), 7(b) and 10(b) are amended as shown above to substitute "SEQ ID NO:5" for "SEQ ID NO:6." Claims 1(b), 7(b) and 10(b) are meant to refer to murine RANK, which as explained above is SEQ ID NO:5, not SEQ ID NO:6. Thus, these amendments do not constitute the addition of new matter.

Claim 6 is amended to correct the inadvertent misspelling of "Paget's" and to delete an inadvertently included word that one skilled in the art would readily recognize as being superfluous. Thus, these amendments do not constitute the addition of new matter.

New Claim 13 is supported throughout the specification, for example, at page 7, line 13, to page 8, line 7. New Claim 14 is supported throughout the specification, for example, in originally filed Claims 2, 7 and 10; at page 3, lines 3-14; at page 4, lines 20-26; page 4, line 37 to page 5, line 5; and in the two excerpts added above by amendment to the specification. New Claim 15 is supported throughout the specification, for example, in originally filed Claims 3, 8 and 11 and at page 4, lines 26-31. New Claim 16 is supported throughout the specification, for example, in originally filed Claims 4, 9 and 12, and at page 3, line 22 to page 4, line 19. New Claims 17-20 are supported, for example, at page 3, lines 31-32, and at page 4, line 18. New Claims 21-24 are supported in the specification, for example, at page 3, lines 3-8. Thus, these new claims do not constitute the addition of new matter.

If the examiner has any questions about this application, he or she is urged to contact the undersigned at her direct dial telephone number given below.

Respectfully submitted,

Diana K. Sheiness

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TITLE

METHOD OF INHIBITING OSTEOCLAST ACTIVITY

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the field of cytokine receptors, and more specifically to cytokine receptor/ligand pairs having osteoclast regulatory activity.

BACKGROUND OF THE INVENTION

RANK (Receptor Activator of NF-κB) and its ligand (RANKL) are a recently-described receptor/ligand pair that play an important role in an immune response. The cloning of RANK and RANKL is described in USSN 08/996,139 and USSN 08/995,659, respectively. It has recently been found that RANKL binds to a protein referred to as osteoprotegerin (OPG), a member of the Tumor Necrosis Factor Receptor (TNFR) family. Yasuda et al. (*Proc. Natl. Acad. Sci.* 95:3597; 1998) expression cloned a ligand for OPG, which they referred to as osteoclastogenesis inhibitory factor. Their work was repeated by Lacey et al. (*Cell* 93:165; 1998). In both cases, the ligand they cloned turned out to be identical to RANKL.

In osteoclastogenesis, the interaction of an osteoblast or stromal cell with an osteoclast precursor leads to the differentiation of the precursor into an osteoclast. OPG was known to inhibit this differentiation. A model has been proposed in which RANKL on the osteoblast or stromal cell surface interacts with a specific receptor on an osteoclast progenitor surface, signaling a differentiation event. OPG effectively blocks the interaction of RANKL with a receptor on osteoclast progenitors *in vitro*, and has been shown to ameliorate the effects of ovariectomy on bone-loss in mice. However, OPG is also known to bind other ligands in the TNF family, which may have a deleterious effect on the activities of such ligands *in vivo*. Moreover, the presence of other ligands that bind OPG in vivo may require high dosages of OPG to be administered in order to have sufficient soluble OPG available to inhibit osteoclastogenesis.

Accordingly, there is a need in the art to identify soluble factors that specifically bind RANKL and inhibit the ability of RANKL to induce osteoclastogenesis without reacting with other ligands.

SUMMARY OF THE INVENTION

The present invention provides processes associated with the use of a novel receptor, referred to as RANK (for receptor activator of NF-κB), that is a member of the TNF receptor superfamily. RANK is a Type I transmembrane protein having 616 amino acid residues, comprising an extracellular domain, transmembrane region and cytoplasmic domain. RANK interacts with various TNF Receptor Associated Factors (TRAFs);

triggering of RANK results in the upregulation of the transcription factor NF-κB, a ubiquitous transcription factor that is most extensively utilized in cells of the immune system.

Soluble forms of the receptor can be prepared and used to interfere with signal transduction through membrane-bound RANK. Inhibition of RANKL-mediated signal transduction will be useful in ameliorating the effects of osteoclastogenesis and osteoclast activity in disease conditions in which there is excess bone break down. Examples of such conditions include osteoporosis, Paget's disease, cancers that may metastasize to bone and induce bone breakdown (i.e., multiple myeloma, breast cancer, some melanomas; see also Mundy, C. *Cancer Suppl.* 80:1546; 1997), and cancers that do not necessarily metastasize to bone, but result in hypercalcemia and bone loss (e.g. squamous cell carcinomas).

Soluble forms of RANK comprise the extracellular domain of RANK or a fragment thereof that binds RANKL. Fusion proteins of RANK may be made to allow preparation of soluble RANK. Examples of such fusion proteins include a RANK/Fc fusion protein, a fusion protein of a zipper moiety (i.e., a leucine zipper), and various tags that are known in the art. Other antagonists of the interaction of RANK and RANKL (i.e., antibodies to RANKL, small molecules) will also be useful in the inventive methods. These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

A novel partial cDNA insert with a predicted open reading frame having some similarity to CD40 was identified and was used to hybridize to colony blots generated from a dendritic cell (DC) cDNA library containing full-length cDNAs. SEQ ID NO:1 shows the nucleotide and amino acid sequence of a predicted full-length protein.

RANK is a member of the TNF receptor superfamily; it most closely resembles CD40 in the extracellular region. RANK is expressed on epithelial cells, some B cell lines, and on activated T cells. However, its expression on activated T cells is late, about four days after activation. This time course of expression coincides with the expression of Fas, a known agent of apoptosis. RANK may act as an anti-apoptotic signal, rescuing cells that express RANK from apoptosis as CD40 is known to do. Alternatively, RANK may confirm an apoptotic signal under the appropriate circumstances, again similar to CD40. RANK and its ligand are likely to play an integral role in regulation of the immune and inflammatory response. The isolation of a DNA encoding RANK is described in USSN 08/996,139, filed December 22 1997, the disclosure of which is

incorporated by reference herein. USSN 08/996,139 describes several forms of RANK that are useful in the present invention.

Soluble RANK comprises the signal peptide and the extracellular domain (residues 1 to 213 of SEQ ID NO:2) or a fragment thereof. Alternatively, a different signal peptide can be substituted for the native leader, beginning with residue 1 and continuing through a residue selected from the group consisting of amino acids 24 through 33 (inclusive) of SEQ ID NO:2. Moreover, fragments of the extracellular domain will also provide soluble forms of RANK.

Fragments can be prepared using known techniques to isolate a desired portion of the extracellular region, and can be prepared, for example, by comparing the extracellular region with those of other members of the TNFR family (of which RANK is a member) and selecting forms similar to those prepared for other family members. Alternatively, unique restriction sites or PCR techniques that are known in the art can be used to prepare numerous truncated forms which can be expressed and analyzed for activity.

Other derivatives of the RANK proteins within the scope of this invention include covalent or aggregative conjugates of the proteins or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α-factor leader).

Protein fusions can comprise peptides added to facilitate purification or identification of RANK proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as that described by Hopp et al., *Bio/Technology* 6:1204 (1988; FLAG™). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein.

Fusion proteins further comprise the amino acid sequence of a RANK linked to an immunoglobulin Fc region. An exemplary Fc region is a human IgG_1 having a nucleotide an amino acid sequence set forth in SEQ ID NO:3. Fragments of an Fc region may also be used, as can Fc muteins. For example, certain residues within the hinge region of an Fc region are critical for high affinity binding to FcγRI. Canfield and Morrison (*J. Exp. Med.* 173:1483; 1991) reported that $Leu_{(234)}$ and $Leu_{(235)}$were critical to high affinity binding of IgG_3 to FcγRI present on U937 cells. Similar results were obtained by Lund et al. (*J. Immunol.* 147:2657, 1991; *Molecular Immunol.* 29:53, 1991). Such mutations, alone or in combination, can be made in an IgG_1 Fc region to decrease the affinity of IgG_1

for FcR. Depending on the portion of the Fc region used, a fusion protein may be expressed as a dimer, through formation of interchain disulfide bonds. If the fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a protein oligomer with as many as four RANK regions.

In another embodiment, RANK proteins further comprise an oligomerizing peptide such as a zipper domain. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Zipper domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for multimerization of the proteins. The zipper domain comprises a repetitive heptad repeat, with four or five leucine, isoleucine or valine residues interspersed with other amino acids. Examples of zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*, also exhibit zipper domains, as does the gene product of the murine proto-oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos* and *jun* comprise zipper domains that preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989). A preferred zipper moiety is that of SEQ ID NO:6 or a fragment thereof. This and other zippers are disclosed in US Patent 5,716,805.

Other embodiments of useful proteins include RANK polypeptides encoded by DNAs capable of hybridizing to the DNA of SEQ ID NO:1 under moderately stringent conditions (prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) to the DNA sequences encoding RANK, or more preferably under stringent conditions (for example, hybridization in 6 X SSC at 63°C overnight; washing in 3 X SSC at 55°C), and other sequences which are degenerate to those which encode the RANK. In one embodiment, RANK polypeptides are at least about 70% identical in amino acid sequence to the amino acid sequence of native RANK protein as set forth in SEQ ID NO:2 for human RANK and NO:6 for murine RANK. In a preferred embodiment, RANK polypeptides are at least about 80% identical in amino acid sequence to the native form of RANK; most preferred polypeptides are those that are at least about 90% identical to native RANK.

Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). For fragments derived from the RANK protein, the identity is calculated based on that portion of the RANK protein that is present in the fragment

The biological activity of RANK analogs or muteins can be determined by testing the ability of the analogs or muteins to bind RANKL, for example as described in the

Examples herein. Suitable assays include, for example, an enzyme immunoassay or a dot blot, and assays that employ cells expressing RANKL. Suitable assays also include, for example, inhibition assays, wherein soluble RANK is used to inhibit the interaction of RANKL with membrane-bound or solid-phase associated RANK (i.e., signal transduction assays). Such methods are well known in the art.

RANKL and RANK are important factors in osteoclastogenesis. RANK is expressed on osteoclasts and interacts with RANK ligand (RANKL) to mediate the formation of osteoclast-like (OCL) multinucleated cells. This was shown by treating mouse bone marrow preparations with M-CSF (CSF-1) and soluble RANKL for 7 days in culture. No additional osteoclastogenic hormones or factors were necessary for the generation of the multinucleated cells. Neither M-CSF nor RANKL alone led to the formation of OCL. The multinucleated cells expressed tartrate resistant acid phosphatase and were positive for [^{125}]- calcitonin binding. The tyrosine kinase c-src was highly expressed in multinucleated OCL and a subset of mononuclear cells as demonstrated by immunofluorescence microscopy. (See Example 2).

Purification of Recombinant RANK

Purified RANK, and homologs or analogs thereof are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a counter structure protein or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying RANK and homologs thereof. For example, a RANK expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a RANK protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing

zipper domain. Monoclonal antibodies against the RANK protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand may also be used to prepare an affinity matrix for affinity purification of RANK.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a RANK composition. Suitable methods include those analogous to the method disclosed by Urdal et al. (*J. Chromatog. 296*:171, 1984). Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification.

Protein synthesized in recombinant culture is characterized by the presence of cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the inventive proteins free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

Uses and Administration of RANK Compositions

The present invention provides methods of using therapeutic compositions comprising a protein and a suitable diluent and carrier. These methods involve the use of therapeutic compositions of RANK or soluble fragments of RANK for regulating an immune or inflammatory response. Further included within the present invention are methods for regulating osteoclast activity by administering therapeutic compositions of RANK or soluble RANK fragments to an individual in amounts sufficient to decrease excess bone resorption. Typically, the individual is inflicted with excess bone resorption and suffers from the effects of hypercalcemia, has symptoms of hypercalcemia, or is suffering a disease that involves excessive bone resorption. In addition to regulating osteoclast activity, the methods described herein are applicable to inhibiting osteoclast

activity, regulating osteoclast generation and inhibiting osteoclast generation in individuals inflicted with excess bone resorption. In connection with the methods described herein, the present invention contemplates the use of RANK in conjunction with soluble cytokine receptors or cytokines, or other osteoclast/osteoblast regulatory molecules.

In connection with the methods described herein, RANK ligand (RANKL) on osteoblasts or stromal cells is known to interact with RANK on osteoclast progenitor surfaces signaling an event that leads to the differentiation of osteoclast precursors into osteoclasts. (See Example 2 below.) Thus, RANK, and in particular soluble forms of RANK, is useful for the inhibition of the RANKL-mediated signal transduction that leads to the differentiation of osteoclast precursors into osteoclasts. Soluble forms of RANK are also useful for the regulation and inhibition of osteoclast activity, e.g. bone resorption. By interfering with osteoclast differentiation, soluble forms of RANK are useful in the amelioration of the effects of osteoclastogenesis in disease conditions in which there is excess bone break down. Such disease conditions include Paget's disease, osteoporosis, and cancer. Many cancers metastasize to bone and induce bone breakdown by locally disrupting normal bone remodeling. Such cancers can be associated with enhanced numbers of osteoclasts and enhanced amount of osteoclastic bone resorption resulting in hypercalcemia. These cancers include, but are not limited to, breast cancer, multiple myeloma, melanomas, lung cancer, prostrate, hematologic, head and neck, and renal. (See Guise et al. *Endocrine Reviews*, 19(1):18-54, 1998.) Soluble forms of RANK can be administered to such cancer patients to disrupt the osteoclast differentiation pathway and result in fewer numbers of osteoclast, less bone resorption, and relief from the negative effects of hypercalcemia.

Other cancers do not metastasize to bone, but are known to act systemically on bone to disrupt bone remodeling and result in hypercalcemia. (See Guise et al. Endocrine Reviews, 19(1):18-54, 1998.) In accordance with this invention, RANKL has been found on the surface of certain squamous cells that do not metastasize to bone but are associated with hypercalcemia. (See Example 3 below) Squamous cells that are associated with hypercalcemia also express M-CSF (CSF-1), a cytokine that, together with RANKL, stimulates the proliferation and differentiation of osteoclast precursors to osteoclasts. In accordance with the present invention, it has been discovered that M-CSF directly upregulates RANK on surfaces of osteoclast precursors. When squamous cells release excessive amounts of CSF-1, increased expression of RANK occurs on the surfaces of osteoclast precursors. Thus, there is a higher probability that RANK will interact with RANKL on osteoblasts or stromal cells to produce increased numbers of osteoclasts, resulting in an enhanced amount of bone break down and hypercalcemia.

In addition to the ameliorating the effects of cancers that metastasize to bone, the present invention provides methods for ameliorating the systemic effects, e.g. hypercalcemia, of cancers that are associated with excess osteoclast activity (e.g. squamous cell carcinomas). Such methods include administering soluble forms of RANK in amounts sufficient to interfere with the RANK/RANKL signal transduction that leads to the differentiation of osteoclast precursors into osteoclasts. Fewer osteoclasts lead to reduced bone resorption and relief from the negative effects of hypercalcemia.

For therapeutic use, purified protein is administered to an individual, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example, RANK protein compositions administered to regulate osteoclast function can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique. Typically, a therapeutic agent will be administered in the form of a composition comprising purified RANK, in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages and concentrations employed.

Ordinarily, the preparation of such protein compositions entails combining the inventive protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and so forth.

Soluble forms of RANK and other RANK antagonists such as antagonistic monoclonal antibodies can be administered for the purpose of inhibiting RANK-induced osteoclastogenesis. It is desirable to inhibit osteoclastogenesis in various disease states in which excess bone loss occurs. Examples include osteoporosis, Pagett's disease, and various cancers. Various animal models of these diseases are known in the art; accordingly, it is a matter of routine experimentation to determine optimal dosages and routes of administration of soluble RANK, first in an animal model and then in human clinical trials.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

EXAMPLE 1

This example describes a plate binding assay useful in comparing the ability of various ligands to bind receptors. The assay is performed essentially as described in Smith et al., Virology 236:316 (1997). Briefly, 96-well microtiter plates are coated with an antibody to human Fc (i.e., polyclonal goat anti human Fc). Receptor/Fc fusion proteins are then added, and after incubation, the plates are washed. Serial dilutions of the ligands are then added. The ligands may be directly labeled (i.e., with ^{125}I), or a detecting reagent that is radioactively labeled may be used. After incubation, the plates are washed, specifically bound ligands are released, and the amount of ligand bound quantified.

Using this method, RANK/Fc and OPG/Fc were bound to 96-well plates. In an indirect method, a RANKL/zipper fusion is detected using a labeled antibody to the zipper moiety. It was found that human OPG/Fc binds mRANKL at 0.05 nM, and human RANK/Fc binds mRANKL at 0.1 nM. These values indicate similar binding affinities of OPG and RANK for RANKL, confirming the utility of RANK as an inhibitor of osteoclast activity in a manner similar to OPG.

EXAMPLE 2

The following describes the formation of osteoclast like cells from bone marrow cell cultures using a soluble RANKL in the form of soluble RANKL/leucine zipper fusion protein (RANKL LZ).

Using RANKL LZ at 1μg/ml, osteoclasts were generated from murine bone marrow (BM) in the presence of CSF-1. These osteoclasts are formed by the fusion of macrophage-like cells and are characterized by their TRAP (tartrate-resistant acid phosphatase) positivity. No $TRAP^+$ cells were seen in cultures containing CSF-1 alone or in cultures containing CSF-1 and TRAIL LZ (a control for the soluble RANKL LZ). Even though human and monkey bone marrow contains more contaminating fibroblasts than murine bone marrow, osteoclasts were generated from murine and monkey bone marrow with the combination of CSF-1 and soluble RANKL LZ. In a dose-response study using murine bone marrow and suboptimal amounts of CSF-1 (40ng/ml), the effects of soluble RANKL LZ plateaued at about 100ng/ml.

The effect of soluble RANKL LZ on proliferation of cells was studied in the same cultures using Alamar Blue. After 5 days, the proliferative response was lower in cultures containing CSF-1 and RANKL LZ than in those containing CSF-1 alone. The supports the observation that soluble RANKL LZ is inducing osteoclast differentiation. When CSF-1 and RANKL LZ are washed out of murine BM cultures at day 7 or 8, cells do not survive if they are recultured in medium or in RANKL LZ alone. In contrast, cells do

survive if recultured in CSF-1. When RANKL LZ was added to these cultures there was no added benefit. Thus, the combination of CSF-1 and RANKL are required for the generation of osteoclast. Additionally, once formed, CSF-1 is sufficient to maintain their survival in culture.

Finally, using human bone marrow, soluble anti-human RANK mAb and immobilized anti-human RANK mAb were compared to RANKL LZ for the generation of osteoclasts in the presence of CSF-1. Immobilized M331 and RANKL LZ were found to be equally effective for osteoclast generation while soluble M331 was superior to both immobilized antibody and RANKL LZ. This confirms that the osteoclast differentiating activity of RANKL is mediated through RANK rather than via an alternative receptor.

Since osteoclasts cannot readily be harvested and analyzed by flow cytometry, ^{125}I-labeled calcitonin binding assays were used to identify osteoclasts (the calcitonin receptor is considered to be an osteoclast-specific marker). Osteoclasts generated from murine BM cultured with CSF-1 and RANKL LZ for 9 days showed binding of radiolabeled calcitonin confirming their osteoclast identity.

EXAMPLE 3

In order to determine RANKL expression by either of two different squamous cell carcinomas, standard Western blot and RT-PCR studies were performed on MH-85 and OKK cells. One of these carcinoma cells, the MH-85 cells, is associated with hypercalcemia.

The results confirmed that MH-85 and OKK squamous cells express RANKL. MH-85 cells, in addition to being linked with hypercalcemia in patients inflicted with this carcinoma, also express M-CSF (CSF-1). It was also determined that CSF-1 upregulates RANK expression on osteoclast precursors. The enhanced amount of CSF-1 in MH-85 type squamous cell cancer patients can lead to an upregulation of RANK and increased RANK interaction with RANKL. Signals transduced by RANK and RANKL interaction result in increased numbers of mature osteoclasts and bone breakdown. Since soluble forms of RANK can inhibit the RANK/RANKL interaction, administering a soluble form of RANK (e.g. the extracellular region of RANK fused to an Fc) to a squamous cell cancer patient provides relief from adverse effects of this cancer, including hypercalcemia.

CLAIMS

We claim:

1. A method of regulating osteoclast activity, the method comprising causing a soluble RANK to bind RANKL.

2. The method of claim 1, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.

3. The method of claim 2, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK

4. The method of claim 3, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG™ tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

5. A method of ameliorating effects of excess bone loss, comprising administering a soluble RANK polypeptide composition to an individual at risk for excess bone loss, and allowing the soluble RANK to bind RANKL and inhibit binding thereof to cells expressing RANK.

6. The method of claim 5, wherein the individual is at risk from or suffers from a condition selected from the group consisting of osteoporosis, Pagett's disease, and bone cancer, and cancers associated with hypercalcemia.

7. The method of claim 5, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.

8. The method of claim 7, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK

9. The method of claim 8, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG™ tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

10. The method of claim 6, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.

11. The method of claim 10, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK

12. The method of claim 11, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG™ tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Immunex Corporation
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Galibert, Laurent

(ii) TITLE OF INVENTION: METHOD OF INHIBITING OSTEOCLAST ACTIVITY

(iii) NUMBER OF SEQUENCES: 6

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #2.0

(vi) CURRENT APPLICATION DATA:
(A) INT'L APPLICATION NUMBER: --to be assigned--
(B) FILING DATE: 13 May 1999
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 34,347
(C) REFERENCE/DOCKET NUMBER: 2874-WO

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(B) TELEFAX: (206)233-0644

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3136 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS

(B) CLONE: FULL LENGTH RANK

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 39..1886

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCGCTGAGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG CGC GCC          53
                                            Met Ala Pro Arg Ala
                                              1               5

CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTG CTC TGC GCG CTG CTC        101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu Leu
                 10                  15                  20

GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG        149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu
             25                  30                  35

AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA        197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly
         40                  45                  50

AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG        245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu
     55                  60                  65

CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA        293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys
 70                  75                  80                  85

TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG        341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val
                 90                  95                 100

GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG        389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly
             105                 110                 115

TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC        437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys
         120                 125                 130

GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA        485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr
     135                 140                 145

GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC        533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser
150                 155                 160                 165

ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA        581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg
                 170                 175                 180

GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT        629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser
             185                 190                 195

CTG CCA GCT AGA AAA CCA CCA AAT GAA CCC CAT GTT TAC TTG CCC GGT        677
Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly
         200                 205                 210
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TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC CTG GTG GCT GCC ATC        725
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    215                 220                 225

ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA GCA CTC ACA GCT AAT        773
Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn
230                 235                 240                 245

TTG TGG CAC TGG ATC AAT GAG GCT TGT GGC CGC CTA AGT GGA GAT AAG        821
Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys
                250                 255                 260

GAG TCC TCA GGT GAC AGT TGT GTC AGT ACA CAC ACG GCA AAC TTT GGT        869
Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly
            265                 270                 275

CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG ACT CTG GAG GAG AAG        917
Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys
        280                 285                 290

ACA TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA GGT GGT GTC TGT CAG        965
Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys Gln
    295                 300                 305

GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGC GAA GAT GCC AGG       1013
Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg
310                 315                 320                 325

ATG CTC TCA TTG GTC AGC AAG ACC GAG ATA GAG GAA GAC AGC TTC AGA       1061
Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg
                330                 335                 340

CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG CCC TCC CAG CCC ACA       1109
Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr
            345                 350                 355

GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT CCT       1157
Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro
        360                 365                 370

TTC TCT GAA CCC CTG GAG GTG GGG GAG AAT GAC AGT TTA AGC CAG TGC       1205
Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys
    375                 380                 385

TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA AGC TGC AAC TGC ACT       1253
Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr
390                 395                 400                 405

GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG TCC TCT GAA AAC TAC       1301
Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr
                410                 415                 420

TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG CAC TGG GCA GCC AGC       1349
Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser
            425                 430                 435

CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC CCT CCT       1397
Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro
        440                 445                 450

GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA AAA CGT GGA CCC TTG       1445
Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro Lys Arg Gly Pro Leu
    455                 460                 465
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Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala Ser
470                 475                 480                 485

AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG GCT GAT GGG AGG CTC      1541
Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg Leu
                490                 495                 500

CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA AGC TCC CCT GGT GGC      1589
Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly Ser Ser Pro Gly Gly
            505                 510                 515

CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC AGT AAC TCC ACG TTC      1637
Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe
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Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val
    535                 540                 545

TAC GTC AGC CAG ACC TCG CAG GAG GGC GCG GCG GCG GCT GCG GAG CCC      1733
Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala Ala Ala Ala Glu Pro
550                 555                 560                 565

ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG CGC CGA GAC TCC TTC      1781
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                570                 575                 580

GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC GGC GGC CCC GAG GGG      1829
Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly
            585                 590                 595

CTG CGG GAG CCG GAG AAG GCC TCG AGG CCG GTG CAG GAG CAA GGC GGG      1877
Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly
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GCC AAG GCT TGAGCGCCCC CCATGGCTGG GAGCCCGAAG CTCGGAGCCA              1926
Ala Lys Ala
    615

GGGCTCGCGA GGGCAGCACC GCAGCCTCTG CCCCAGCCCC GGCCACCCAG GGATCGATCG    1986

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CACTGGGGCA GATGTCTCCC CTGCCACTCC TCAAACTCGC AGCAGTAATT TGTGGCACTA    2166

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GTTTTCTCAA AAATTCTCCT AAAGGTGAGG GTCTCTTTCT TTTCTCTTTT CCTTTTTTTT    2346

TTCTTTTTTT GGCAACCTGG CTCTGGCCCA GGCTAGAGTG CAGTGGTGCG ATTATAGCCC    2406

GGTGCAGCCT CTAACTCCTG GGCTCAAGCA ATCCAAGTGA TCCTCCCACC TCAACCTTCG    2466

GAGTAGCTGG GATCACAGCT GCAGGCCACG CCCAGCTTCC TCCCCCCGAC TCCCCCCCCC    2526

CAGAGACACG GTCCCACCAT GTTACCCAGC CTGGTCTCAA ACTCCCCAGC TAAAGCAGTC    2586

CTCCAGCCTC GGCCTCCCAA AGTACTGGGA TTACAGGCGT GAGCCCCCAC GCTGGCCTGC    2646
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TTTACGTATT TTCTTTTGTG CCCCTGCTCA CAGTGTTTTA GAGATGGCTT TCCCAGTGTG    2706

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AGTTGCTAGG AACATGTGGT GGGACTTTCA TATTCTGAAA AATGTTCTAT ATTCTCATTT    2826

TTCTAAAAGA AAGAAAAAAG GAAACCCGAT TTATTTCTCC TGAATCTTTT TAAGTTTGTG    2886

TCGTTCCTTA AGCAGAACTA AGCTCAGTAT GTGACCTTAC CCGCTAGGTG GTTAATTTAT    2946

CCATGCTGGC AGAGGCACTC AGGTACTTGG TAAGCAAATT TCTAAAACTC CAAGTTGCTG    3006

CAGCTTGGCA TTCTTCTTAT TCTAGAGGTC TCTCTGGAAA AGATGGAGAA AATGAACAGG    3066

ACATGGGGCT CCTGGAAAGA AAGGGCCCGG GAAGTTCAAG GAAGAATAAA GTTGAAATTT    3126

TAAAAAAAAA                                                           3136
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 616 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ala Pro Arg Ala Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
  1               5                  10                  15

Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
            20                  25                  30

Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
        35                  40                  45

Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
    50                  55                  60

Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
65                  70                  75                  80

Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
                85                  90                  95

Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
            100                 105                 110

Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
        115                 120                 125

Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
    130                 135                 140

Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
145                 150                 155                 160

Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
                165                 170                 175
```

```
Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
            180                 185                 190

Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
        195                 200                 205

Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
    210                 215                 220

Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
225                 230                 235                 240

Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
                245                 250                 255

Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
            260                 265                 270

Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
        275                 280                 285

Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
    290                 295                 300

Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
305                 310                 315                 320

Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
                325                 330                 335

Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
            340                 345                 350

Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
        355                 360                 365

Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
    370                 375                 380

Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
385                 390                 395                 400

Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
                405                 410                 415

Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
            420                 425                 430

His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
        435                 440                 445

Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro
    450                 455                 460

Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro
465                 470                 475                 480

Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly
                485                 490                 495

Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly
            500                 505                 510
```

```
Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn
        515                 520                 525

Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly
    530                 535                 540

Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala
545                 550                 555                 560

Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala
                565                 570                 575

Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys
            580                 585                 590

Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val
        595                 600                 605

Gln Glu Gln Gly Gly Ala Lys Ala
    610                 615
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human

(vii) IMMEDIATE SOURCE:
(B) CLONE: IgG1 Fc mutein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
  1               5                  10                  15

Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
             20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
         35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
     50                  55                  60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 65                  70                  75                  80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125
```

```
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145                 150                 155                 160

His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1878 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Murine

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Murine Fetal Liver Epithelium
(B) CLONE: muRANK

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
ATG GCC CCG CGC GCC CGG CGG CGC CGC CAG CTG CCC GCG CCG CTG CTG   48
Met Ala Pro Arg Ala Arg Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
  1               5                  10                  15

GCG CTC TGC GTG CTG CTC GTT CCA CTG CAG GTG ACT CTC CAG GTC ACT   96
Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
             20                  25                  30

CCT CCA TGC ACC CAG GAG AGG CAT TAT GAG CAT CTC GGA CGG TGT TGC  144
Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
         35                  40                  45
```

```
AGC AGA TGC GAA CCA GGA AAG TAC CTG TCC TCT AAG TGC ACT CCT ACC  192
Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
     50                  55                  60

TCC GAC AGT GTG TGT CTG CCC TGT GGC CCC GAT GAG TAC TTG GAC ACC  240
Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
 65                  70                  75                  80

TGG AAT GAA GAA GAT AAA TGC TTG CTG CAT AAA GTC TGT GAT GCA GGC  288
Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly
                 85                  90                  95

AAG GCC CTG GTG GCG GTG GAT CCT GGC AAC CAC ACG GCC CCG CGT CGC  336
Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
            100                 105                 110

TGT GCT TGC ACG GCT GGC TAC CAC TGG AAC TCA GAC TGC GAG TGC TGC  384
Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
        115                 120                 125

CGC AGG AAC ACG GAG TGT GCA CCT GGC TTC GGA GCT CAG CAT CCC TTG  432
Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu
    130                 135                 140

CAG CTC AAC AAG GAT ACG GTG TGC ACA CCC TGC CTC CTG GGC TTC TTC  480
Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145                 150                 155                 160

TCA GAT GTC TTT TCG TCC ACA GAC AAA TGC AAA CCT TGG ACC AAC TGC  528
Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
                165                 170                 175

ACC CTC CTT GGA AAG CTA GAA GCA CAC CAG GGG ACA ACG GAA TCA GAT  576
Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
            180                 185                 190

GTG GTC TGC AGC TCT TCC ATG ACA CTG AGG AGA CCA CCC AAG GAG GCC  624
Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala
        195                 200                 205

CAG GCT TAC CTG CCC AGT CTC ATC GTT CTG CTC CTC TTC ATC TCT GTG  672
Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Leu Phe Ile Ser Val
    210                 215                 220

GTA GTA GTG GCT GCC ATC ATC TTC GGC GTT TAC TAC AGG AAG GGA GGG  720
Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Tyr Arg Lys Gly Gly
225                 230                 235                 240

AAA GCG CTG ACA GCT AAT TTG TGG AAT TGG GTC AAT GAT GCT TGC AGT  768
Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser
                245                 250                 255

AGT CTA AGT GGA AAT AAG GAG TCC TCA GGG GAC CGT TGT GCT GGT TCC  816
Ser Leu Ser Gly Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser
            260                 265                 270

CAC TCG GCA ACC TCC AGT CAG CAA GAA GTG TGT GAA GGT ATC TTA CTA  864
His Ser Ala Thr Ser Ser Gln Gln Glu Val Cys Glu Gly Ile Leu Leu
        275                 280                 285

ATG ACT CGG GAG GAG AAG ATG GTT CCA GAA GAC GGT GCT GGA GTC TGT  912
Met Thr Arg Glu Glu Lys Met Val Pro Glu Asp Gly Ala Gly Val Cys
    290                 295                 300
```

```
GGG CCT GTG TGT GCG GCA GGT GGG CCC TGG GCA GAA GTC AGA GAT TCT  960
Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
305                 310                 315                 320

AGG ACG TTC ACA CTG GTC AGC GAG GTT GAG ACG CAA GGA GAC CTC TCG 1008
Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
                325                 330                 335

AGG AAG ATT CCC ACA GAG GAT GAG TAC ACG GAC CGG CCC TCG CAG CCT 1056
Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
            340                 345                 350

TCG ACT GGT TCA CTG CTC CTA ATC CAG CAG GGA AGC AAA TCT ATA CCC 1104
Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
        355                 360                 365

CCA TTC CAG GAG CCC CTG GAA GTG GGG GAG AAC GAC AGT TTA AGC CAG 1152
Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
    370                 375                 380

TGT TTC ACC GGG ACT GAA AGC ACG GTG GAT TCT GAG GGC TGT GAC TTC 1200
Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
385                 390                 395                 400

ACT GAG CCT CCG AGC AGA ACT GAC TCT ATG CCC GTG TCC CCT GAA AAG 1248
Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
                405                 410                 415

CAC CTG ACA AAA GAA ATA GAA GGT GAC AGT TGC CTC CCC TGG GTG GTC 1296
His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
            420                 425                 430

AGC TCC AAC TCA ACA GAT GGC TAC ACA GGC AGT GGG AAC ACT CCT GGG 1344
Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
        435                 440                 445

GAG GAC CAT GAA CCC TTT CCA GGG TCC CTG AAA TGT GGA CCA TTG CCC 1392
Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
    450                 455                 460

CAG TGT GCC TAC AGC ATG GGC TTT CCC AGT GAA GCA GCA GCC AGC ATG 1440
Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
465                 470                 475                 480

GCA GAG GCG GGA GTA CGG CCC CAG GAC AGG GCT GAT GAG AGG GGA GCC 1488
Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
                485                 490                 495

TCA GGG TCC GGG AGC TCC CCC AGT GAC CAG CCA CCT GCC TCT GGG AAC 1536
Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
            500                 505                 510

GTG ACT GGA AAC AGT AAC TCC ACG TTC ATC TCT AGC GGG CAG GTG ATG 1584
Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
        515                 520                 525

AAC TTC AAG GGT GAC ATC ATC GTG GTG TAT GTC AGC CAG ACC TCG CAG 1632
Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
    530                 535                 540

GAG GGC CCG GGT TCC GCA GAG CCC GAG TCG GAG CCC GTG GGC CGC CCT 1680
Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
545                 550                 555                 560
```

```
GTG CAG GAG GAG ACG CTG GCA CAC AGA GAC TCC TTT GCG GGC ACC GCG  1728
Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
                565                 570                 575

CCG CGC TTC CCC GAC GTC TGT GCC ACC GGG GCT GGG CTG CAG GAG CAG  1776
Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
            580                 585                 590

GGG GCA CCC CGG CAG AAG GAC GGG ACA TCG CGG CCG GTG CAG GAG CAG  1824
Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
        595                 600                 605

GGT GGG GCG CAG ACT TCA CTC CAT ACC CAG GGG TCC GGA CAA TGT GCA  1872
Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
    610                 615                 620

GAA TGA                                                         1878
Glu
625
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ala Pro Arg Ala Arg Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
  1               5                  10                  15

Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
            20                  25                  30

Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
        35                  40                  45

Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
    50                  55                  60

Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
65                  70                  75                  80

Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly
                85                  90                  95

Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
            100                 105                 110

Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
        115                 120                 125

Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu
    130                 135                 140

Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145                 150                 155                 160

Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
                165                 170                 175
```

```
Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
            180                 185                 190

Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala
        195                 200                 205

Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Leu Phe Ile Ser Val
    210                 215                 220

Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Tyr Arg Lys Gly Gly
225                 230                 235                 240

Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser
                245                 250                 255

Ser Leu Ser Gly Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser
            260                 265                 270

His Ser Ala Thr Ser Ser Gln Gln Glu Val Cys Glu Gly Ile Leu Leu
        275                 280                 285

Met Thr Arg Glu Glu Lys Met Val Pro Glu Asp Gly Ala Gly Val Cys
    290                 295                 300

Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
305                 310                 315                 320

Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
                325                 330                 335

Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
            340                 345                 350

Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
        355                 360                 365

Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
    370                 375                 380

Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
385                 390                 395                 400

Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
                405                 410                 415

His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
            420                 425                 430

Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
        435                 440                 445

Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
    450                 455                 460

Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
465                 470                 475                 480

Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
                485                 490                 495

Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
            500                 505                 510
```

```
Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
        515                 520                 525

Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
    530                 535                 540

Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
545                 550                 555                 560

Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
                565                 570                 575

Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
            580                 585                 590

Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
        595                 600                 605

Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
    610                 615                 620

Glu
625
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile
  1               5                  10                  15

Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
            20                  25                  30

Arg
```

Immunex Corporation Docket No.: 2874-B

DECLARATION OF INVENTORS

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD OF INHIBITING OSTEOCLAST ACTIVITY

the specification of which is filed herewith.

I hereby state that I have reviewed and understand the contents of said specification, including the claims that are presented in the Preliminary Amendment that is being submitted with the application. I acknowledge the duty to disclose information that is known to me and material to patentability of the subject claimed invention in accordance with 37 C.F.R. §1.56.

(X) I hereby claim the benefit under 35 U.S.C. §120 of the United States application(s) and PCT international application(s) designating the United States that are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) or PCT international application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

Application No.	Filed
PCT/US99/10588	May 13, 1999
USSN 08/966,139	December 22, 1997 (now US Patent 6,017,729)

(X) I hereby claim the benefit under 35 U.S.C. §119(e) of the United States provisional patent application(s) listed below:

Application No.	Filed
60/085,487	May 14, 1998
60/110,836	December 3, 1998
60/064,671	October 14, 1997
60/077,181	March 7, 1997
60/059,978	December 23, 1996

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature__________ Date Nov. 3, 2000

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Citizenship	U.S.

Signature__________ Date Nov. 3rd, 2000

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